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Supplementary Materials and Methods

Additional exclusion criteria:

Additionally, participants with a history of major depression or history of substance abuse in the past year were excluded. None of the ME/CFS cases were receiving antimicrobial treatment or any other specific treatment directed at their ME/CFS. ME/CFS cases were also excluded if they had received long-term (one month or longer) antivirals during the previous year or antibiotics during the previous 3 months to the date of blood collection. None of the controls had received antimicrobial treatment for at least 30 days prior to enrollment

Nonspecific Binding:

Summary: Initially we fit GME regression models that were limited to candidate predictor variables for disease severity category, sex, race, and age, with pMFI as outcome. In the course of our thorough evaluation of the quality of the fit of this initial regression model to the pMFI data, a discovery was made (T.H.H., second author on this article) that the residuals (i.e., variation in pMFI unexplained by fit of regression model; see Figure S1 below) contained strong, unexplained variation not associated with disease severity category, sex, race, and age. We subsequently identified that nonspecific binding was strongly associated with this unexplained structure in the pMFI data and, on this basis, added nonspecific binding as a covariate to the final regression model.

Details: Please refer to Figure S1 which graphically summarizes the steps of this analysis.

Step 1: Initially we fit GME regression models that were limited to candidate predictor variables for disease severity category, sex, race, and age, with pMFI as outcome, separately for each cytokine.

Step 2: Regression residuals from all 51 cytokines were collated into a multivariate dataset with one column for each cytokine and one row for each participant.

Step 3: These multivariate residual data were subjected to a robust (minimum-volume ellipsoid), principal components analysis (rPCA) (1), separately in cases and controls. Principal components analysis extracts weighted combinations of the input variables (here the 51 cytokines' residuals) such that the first principal component explains the largest proportion of the total variance, the second principal component explains the second largest proportion of the total variance and is uncorrelated in the sample with the first principal component, etc. (2).

Using the methods very similar to those detailed in Holmes and He (3) (Section 3.6), we identified that the first three principal components represented structure (i.e., variation in excess of noise levels) in the pMFI residuals. Nearly all of this structure is concentrated in the first principal component, which has the largest variance (eigenvalue; Figure S1 top right). That is, the first principal component accounts for most of the variance in the pMFI residuals after adjustment for disease severity category, sex, race, and age. Importantly, correlation between nonspecific binding and these structural principal components is statistically significant and variable in strength and direction, as shown in the following table of Pearson correlation coefficient estimates (r_p).

Correlation between Nonspecific Binding (CHEX4 pMFI) and First Three Principal Components of Regression Residuals

Group	Principal Component 1st	2nd	3rd
Cases	r_p = -0.66 (p < 0.0001)	r_p = -0.18 (p = 0.0163)	r_p = -0.39 (p < 0.0001)
Controls	r_p = -0.67 (p < 0.0001)	r_p = -0.06 (p = 0.2260)	r_p = 0.38 (p < 0.0001)

This result suggests that nonspecific binding is centrally associated with unexplained structure in cytokine pMFI levels after adjustment for disease severity, age, sex and race. Furthermore, *we discovered that nonspecific binding is correlated with pMFI levels for most cytokines*. Finally, *nonspecific binding was also found to be correlated with half of the predictor variables, including case status*, as shown in the following table of point-biserial partial [Partialing ("adjustment") is with respect to other covariates shown in this table] correlation coefficient estimates (r_b).

Partial Correlation between Nonspecific Binding (CHEX4 pMFI) and Covariates

Case Status	Age	Male Sex	White Race
r_b = -0.12 (p = 0.0028)	r_b = -0.21 (p < 0.0001)	r_b = -0.05 (p = 0.2671)	r_b =0.01 (p =0.8763)

Taken altogether, the association of nonspecific binding with outcomes (pMFI) and predictors (case status and age) allows for the possibility that nonspecific binding is *an omitted predictor variable* that*, in its absence, may be introducing bias into regression parameter estimates* (4). The size and sign of the correlation coefficient for case status (-0.12) indicate that nonspecific binding is slightly lower in cases. Moreover, the multiple components of residual structure (first three principal components) admits the possibility that not one but multiple omitted variables exist, and given their correlation with nonspecific binding (above table of Pearson correlation estimates), *nonspecific binding may serve as a useful surrogate for these multiple omitted variables*. A graphical illustration of regression adjustment for nonspecific binding is provided in Figure S2.

At this time, the specific nature and cause(s) of this nonspecific binding phenomenon are incompletely understood. Nonspecific binding might represent binding by compounds completely unrelated to the biology of the individual (i.e., assay artifact). Variable degree of specificity of the assay's particular antibody pair for a particular cytokine may partially explain why association with CHEX4 (nonspecific binding) varies among

cytokines. The weak correlation between nonspecific binding and case- control status could be attributable to an undiagnosed comorbidity that is imbalanced between cases and controls (5).

Subsequent work will continue to explore the remaining, unidentified structure in the residuals, possibly identifying additional specific omitted variables and/or performing analyses that also adjust for *all* unidentified residual structure (6, 7); however, present adjustment was restricted to the solidly identified omitted variable (nonspecific binding).

Technical Details of Regression Modeling and Correlation Analyses

For completeness of reporting and for those specifically interested in further technical specifications, we offer these additional details.

1) Analysis of ME/CFS Cases vs. Healthy Controls (case versus control means comparisons): We employed pseudo-likelihood ratio testing (8)2 to compare pMFI means between a) each disease severity group versus control and b) the equally-weighted average across all three severity groups versus control.

2) Analysis of ME/CFS Cases by disease severity (primary analysis trends in pMFI means across case severity levels): Linear and curvilinear trends in pMFI means were estimated across the sequence of mild, moderate, and severe disease. Orthogonal linear (linear trend) and quadratic (curvilinear trend) polynomial contrasts were constructed across this severity sequence; and the null hypothesis that each contrast was zero was assessed using pseudo-likelihood ratio testing. For an introduction to orthogonal polynomial contrasts, see (9) (pp. 677-683).

3) Data-driven GME regression modeling: In GME estimation, each regression coefficient estimate is a weighted sum of the "support" values that are supplied by the analyst. We employed especially wide, dense supports (e.g., [-50, -40,…, 40, 50]) for regression coefficients to minimize completely any restrictions on possible values for estimates of regression coefficients in (10) (page 138), thereby making estimates of effects (e.g., case vs. control) as data-driven as possible.

4) Robustness of regression findings to choice of estimation method: We also fit the regression model for the secondary analysis using ordinary least squares (OLS) with allowance for differences in variances (11) among severity categories. An OLS estimation (or closely allied) method was used in a recent study addressing cytokine profiles in ME/CFS patients (12). The normal-distribution assumption for hypothesis testing from OLS parameter estimates relaxes with increasing sample size under very general conditions (13, 14). Findings from GME and OLS estimation are similar (available upon request).

5) Spearman partial correlation analyses: Four sets of Spearman rank correlation coefficients between cytokine pMFI and fatigue duration were estimated: a) without any covariate adjustment, b) with adjustment for age, c) with adjustment for age and CHFX4 pMFI, and d) with adjustment for age, CHFX4 pMFI, sex

and race. Age and CHEX4 pMFI were partialed out per Daniel W.W. (15) (pp. 395-396). Fatigue duration, cytokine pMFI, age, and CHEX4 pMFI were each regressed on race and sex; and those regression residuals were used in the partial correlation analysis adjusted for age, CHEX4 pMFI, sex, and race.

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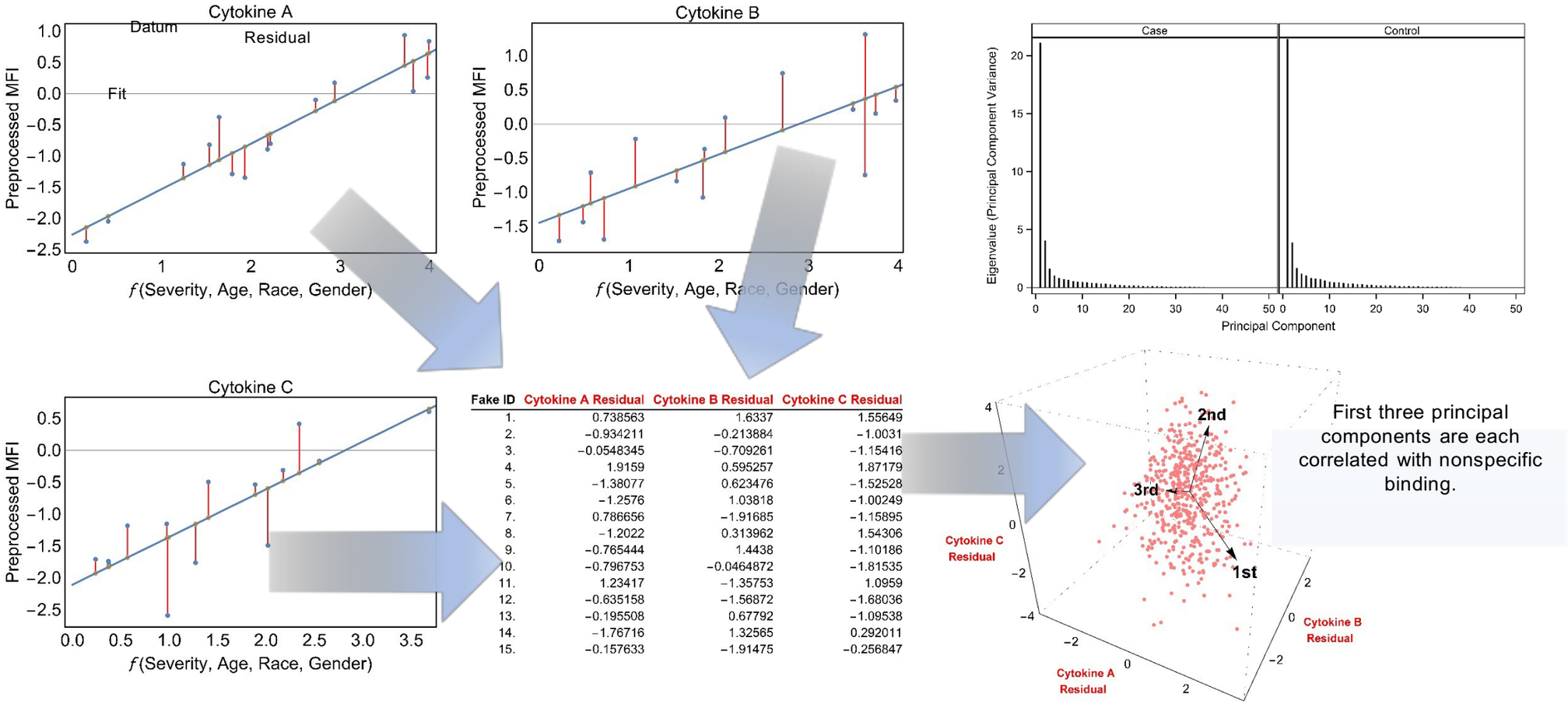
Figure S1. Schematic of statistical workflow for nonspecific binding discovery. Step 1: Separately by cytokine (e.g. Cytokine A, B, C, in this figure for illustration purposes), pMFI was regressed on ME/CFS severity, age, sex and race. Step 2: Residuals (i.e., variation in pMFI unexplained by regression model) were collated into a multivariate dataset. Step 3: A robust principal components analysis was performed on these multivariate residual data.[3] First, second and third principal components represent structure (as opposed to noise) in multivariate (i.e., all 51 cytokines) residual data, as revealed in their eigenvalues (an eigenvalue is the variance of the principal component; top right of schematic. A wide range in sizes of eigenvalues suggests that the residual data may contain signal rather than noise with that signal concentrated in the first few principal components.



Fake ID	Cytokine A Residual	Cytokine B Residual	Cytokine C Residual
1.	0.738563	1.6337	1.55649
2.	−0.934211	−0.213884	−1.0031
3.	−0.0548345	−0.709261	−1.15416
4.	1.9159	0.595257	1.87179
5.	−1.38077	0.623476	−1.52528
6.	−1.2576	1.03818	−1.00249
7.	0.786656	−1.91685	−1.15895
8.	−1.2022	0.313962	1.54306
9.	−0.765444	1.4438	−1.10186
10.	−0.796753	−0.0464872	−1.81535
11.	1.23417	−1.35753	1.0959
12.	−0.635158	−1.56872	−1.68036
13.	−0.195508	0.67792	−1.09538
14.	−1.76716	1.32565	0.292011
15.	−0.157633	−1.91475	−0.256847

[3] Only three cytokines shown here for illustrative purposes. Actual dimensionality of multivariate residual data and principal components space was 51.

Figure S2. An example of regression adjustment for nonspecific binding in study participants. Red and blue denote before and after adjustment. Points are individual data (original and adjusted) and lines are fit of linear regressions. pMFI = pre-processed median fluorescence intensity, as described in "Statistical Analysis" section of main text. Adjustment is shown for pMFI of IFN-β. Regression adjustment leverages information across all data in the sample toremove a highly stable estimate of fluorescence distortion by nonspecific binding. In results presented in main text, in addition to nonspecific binding, regression analysis simultaneously adjusted for disease severity category (control, mild, moderate and severe), sex, race, age, pMFI of the nonspecific binding control (CHEX4), and, to allow for the possibility that covariate effects differ between cases and controls, interaction terms between case status and each covariate (sex, race, age, and pMFI of the nonspecific binding control).

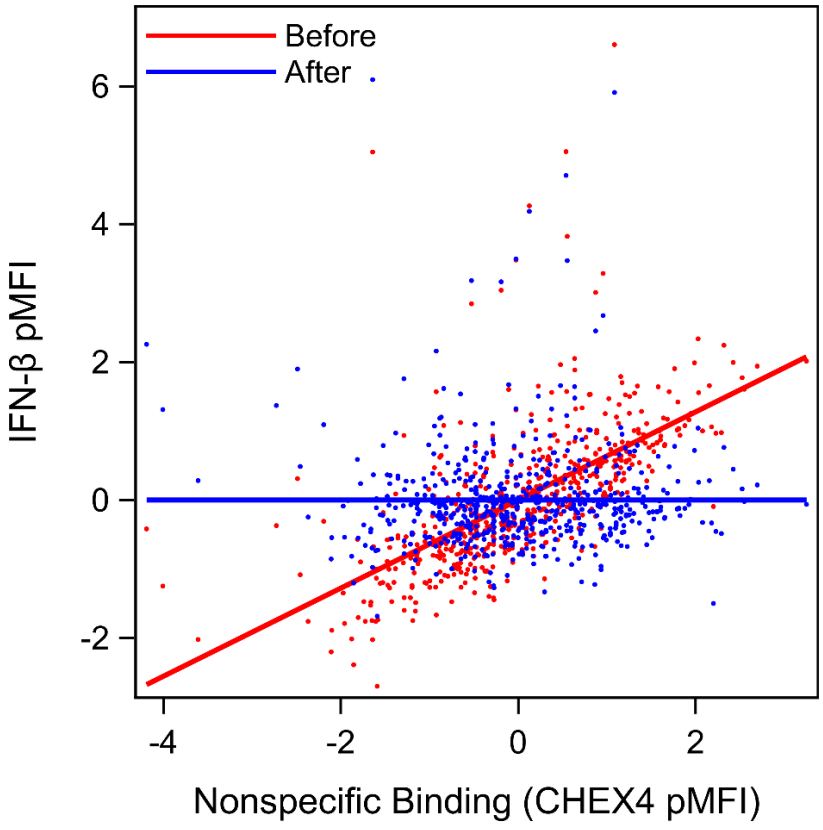


Figure 53. Association of Age and Fatigue Duration in 192 ME/CFS patients. Diagonal line represents maximum possible duration based on patient's age (e.g. a patient of age 40 years has maximum possible duration of 40 years).

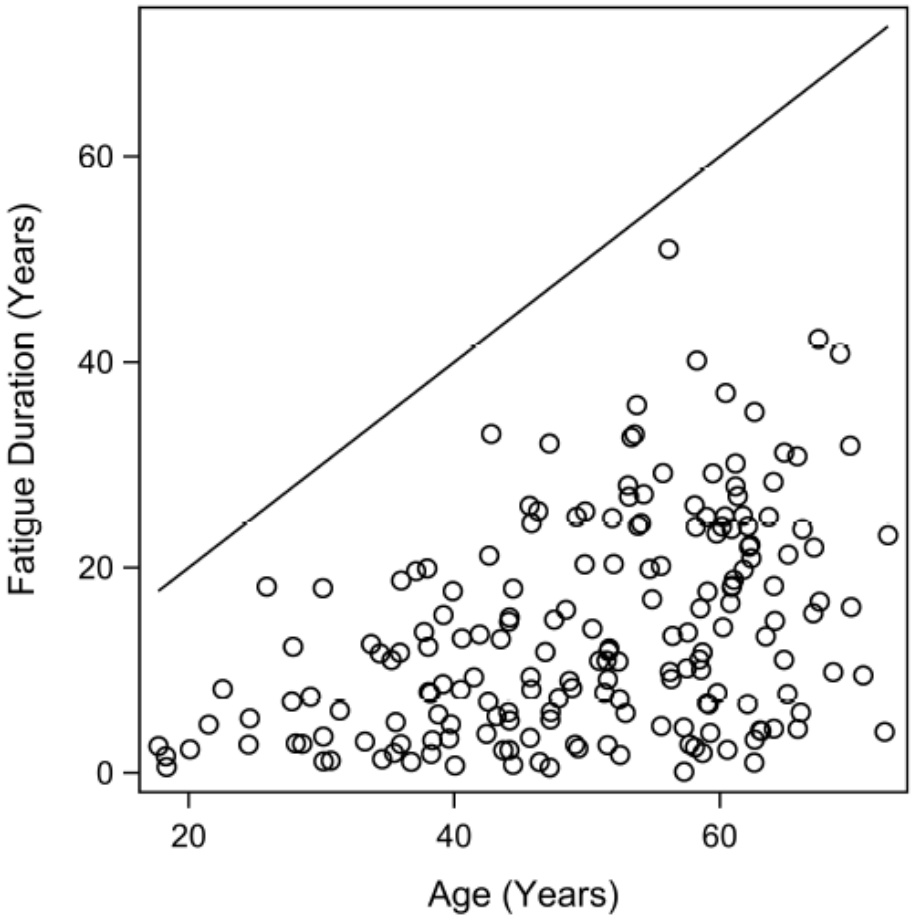


Figure S4. Association of Fatigue Duration and Disease Severity (MFI-20) in 192 ME/CFS patients. Spearman's correlation r_s between fatigue duration (years) and disease severity (MFI-20 total score) was estimated to be $r_s \approx 0.10$ ($p = 0.17$) and $r_s \approx 0.07$ ($p = 0.37$) with and without, respectively, adjustment for age. Open circles are observed data.

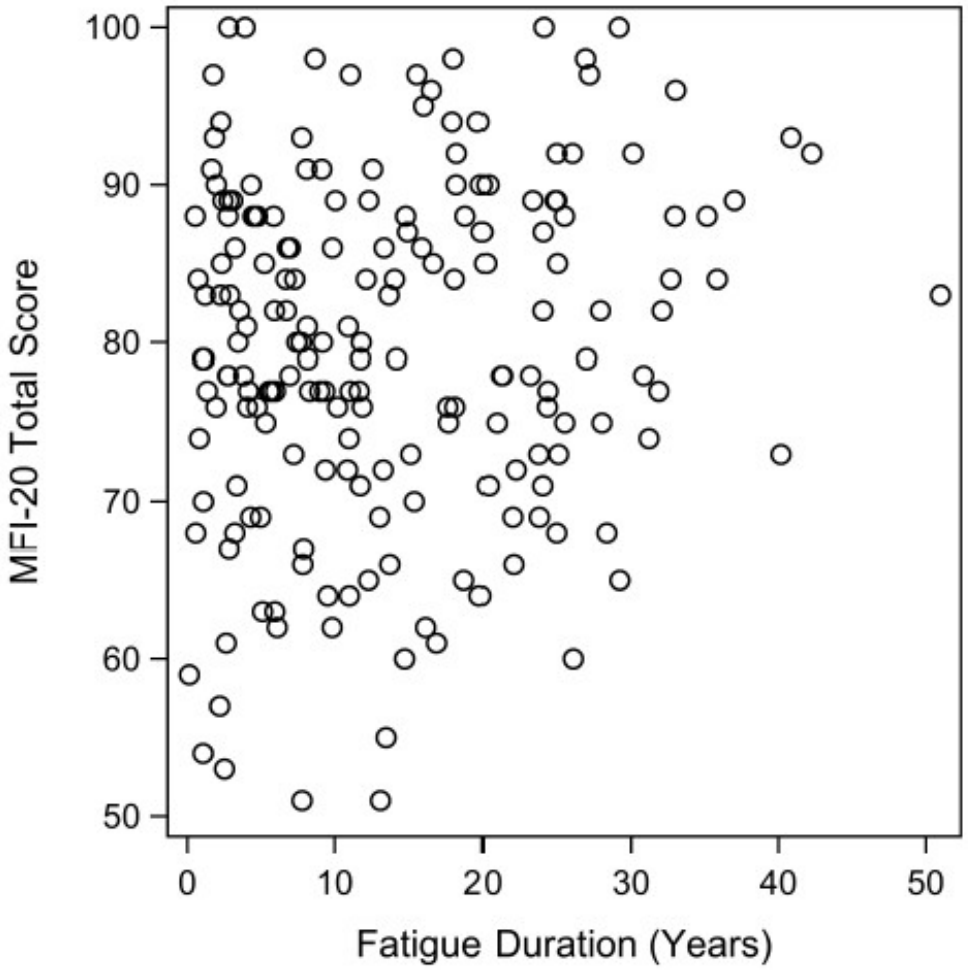


Table S1. Spearman correlation estimates between cytokine levels (pMFI) and fatigue duration, in ME/CFS patients. Analysis was then controlled for age, sex, race and nonspecific binding. Statistically significant ($p < 0.05$) results are in bold. A negative Spearman correlation estimate indicates a reduction in pMFI with increased fatigue duration. pMFI = Pre-processed median fluorescence intensity.

Cytokine	Spearman Correlation Before Controlling for any Variable	Spearman Correlation Before Controlling for any Variable p-value	Spearman Correlation Controlling for Age	Spearman Correlation Controlling for Age p-value	Spearman Correlation Controlling for Age and Nonspecific Binding	Spearman Correlation Controlling for Age and Nonspecific Binding p-value	Spearman Correlation Controlling for Age, Nonspecific Binding, Race and Sex	Spearman Correlation Controlling for Age, Nonspecific Binding, Race and Sex p-value
CCL2	-0.08254	0.2601	-0.03930	0.5933	-0.04297	0.5603	-0.03658	0.6258
CCL3	-0.10124	0.1669	-0.05857	0.4259	-0.06120	0.4066	-0.06355	0.3967
CCL4	-0.12551	0.0861	-0.08175	0.2660	-0.08826	0.2310	-0.09503	0.2045
CCL5	-0.05734	0.4344	0.03329	0.6511	0.03328	0.6520	0.04352	0.5619
CCL7	-0.09544	0.1926	-0.04253	0.5633	-0.04529	0.5393	-0.04322	0.5646
CCL11	-0.06231	0.3956	-0.00050	0.9945	0.00037	0.9960	-0.01489	0.8428
CD40L	-0.12047	0.0996	-0.03382	0.6459	-0.03516	0.6338	-0.05261	0.4830
CXCL1	-0.15158	**0.0378**	-0.04570	0.5345	-0.05278	0.4743	-0.06066	0.4186
CXCL5	-0.00308	0.9665	0.02189	0.7662	0.02320	0.7533	0.02835	0.7056
CXCL9	-0.08373	0.2533	-0.15184	**0.0380**	-0.16239	**0.0268**	-0.18624	**0.0123**
CXCL10	-0.12863	0.0785	-0.09002	0.2205	-0.10054	0.1721	-0.11148	0.1362
FASL	-0.11169	0.1270	0.00573	0.9379	0.00617	0.9334	-0.00082	0.9913
FGF-basic	-0.09959	0.1739	0.01053	0.8862	0.01176	0.8734	0.00997	0.8943
G-CSF	-0.10894	0.1367	-0.02561	0.7279	-0.02828	0.7015	-0.04328	0.5640
GM-CSF	-0.09009	0.2189	0.00160	0.9826	0.00286	0.9691	0.00013	0.9986
HGF	-0.11859	0.1051	-0.04836	0.5110	-0.05227	0.4786	-0.05278	0.4816
ICAM1	0.04236	0.5638	-0.01505	0.8381	-0.01495	0.8395	-0.03902	0.6030
IFN-α	-0.09661	0.1872	-0.01102	0.8810	-0.01102	0.8813	-0.02894	0.6998
IFN-β	-0.14518	**0.0468**	-0.03212	0.6625	-0.04064	0.5818	-0.04087	0.5859

Cytokine	Spearman Correlation before controlling for any variable	Spearman Correlation before controlling for any variable p-value	Spearman Correlation Controlling for Age	Spearman Correlation Controlling for Age p-value	Spearman Correlation Controlling for Age and Nonspecific Binding	Spearman Correlation Controlling for Age and Nonspecific Binding p-value	Spearman Correlation Controlling for Age, Nonspecific Binding, Race and Sex*	Spearman Correlation Controlling for Age, Nonspecific Binding, Race and Sex* p-value
IFN-γ	-0.13040	0.0745	-0.05047	0.4928	-0.05870	0.4261	-0.06892	0.3579
IL-1RA	-0.14499	**0.0471**	-0.08074	0.2720	-0.08491	0.2492	-0.10787	0.1495
IL-1α	-0.15230	**0.0369**	-0.05189	0.4806	-0.06451	0.3817	-0.02825	0.7066
IL-1β	-0.08826	0.2284	0.04060	0.5811	0.06727	0.3616	0.06241	0.4052
IL-2	-0.16472	**0.0239**	-0.02999	0.6836	-0.03566	0.6289	-0.02889	0.7002
IL-4	-0.09395	0.1997	-0.02488	0.7354	-0.02901	0.6943	-0.03624	0.6291
IL-5	-0.09020	0.2183	0.00045	0.9951	0.00163	0.9824	-0.01017	0.8922
IL-6	-0.06022	0.4116	0.06067	0.4094	0.08121	0.2705	0.08112	0.2790
IL-7	-0.06111	0.4048	-0.01564	0.8318	-0.01653	0.8228	-0.01973	0.7926
IL-8	-0.15379	**0.0351**	-0.04094	0.5780	-0.04249	0.5647	-0.04895	0.5141
IL-10	-0.10671	0.1450	-0.05044	0.4930	-0.05714	0.4386	-0.04816	0.5209
IL-12p40	-0.10398	0.1556	-0.02457	0.7386	-0.02709	0.7136	-0.03755	0.6168
IL-12p70	-0.09731	0.1840	-0.01488	0.8399	-0.01631	0.8251	-0.01780	0.8126
IL-13	-0.09088	0.2149	-0.00769	0.9168	-0.00814	0.9122	-0.00888	0.9059
IL-15	-0.14758	**0.0433**	-0.00317	0.9656	-0.00284	0.9693	-0.01142	0.8790
IL-17	-0.09409	0.1990	0.01029	0.8888	0.01157	0.8755	0.01495	0.8421
IL-17F	-0.12727	0.0818	-0.03064	0.6772	-0.03545	0.6310	-0.05166	0.4910
Leptin	0.09354	0.2017	0.06014	0.4135	0.06032	0.4135	0.02870	0.7021
LIF	-0.10847	0.1384	-0.04048	0.5823	-0.04595	0.5334	-0.05215	0.4869
M-CSF	-0.09008	0.2189	0.00559	0.9394	0.00647	0.9302	-0.01147	0.8785
NGF	-0.11001	0.1329	-0.01421	0.8469	-0.01714	0.8164	-0.02131	0.7765
PAI-1	-0.05511	0.4525	0.04522	0.5389	0.04560	0.5366	0.02688	0.7202
PDGF-BB	0.03168	0.6661	0.01840	0.8026	0.01900	0.7969	0.04226	0.5732

Cytokine	Spearman Correlation before controlling for any variable	Spearman Correlation before controlling for any variable p-value	Spearman Correlation Controlling for Age	Spearman Correlation Controlling for Age p-value	Spearman Correlation Controlling for Age and Nonspecific Binding	Spearman Correlation Controlling for Age and Nonspecific Binding p-value	Spearman Correlation Controlling for Age, Nonspecific Binding, Race and Sex*	Spearman Correlation Controlling for Age, Nonspecific Binding, Race and Sex* p-value
Resistin	-0.13532	0.0641	-0.05130	0.4856	-0.05271	0.4749	-0.08439	0.2600
SCF	-0.12025	0.1002	-0.05167	0.4825	-0.05928	0.4216	-0.07464	0.3193
TGF-α	-0.14741	**0.0435**	-0.01458	0.8430	-0.01629	0.8253	-0.02785	0.7105
TGF-β	-0.19175	**0.0084**	-0.03755	0.6099	-0.04796	0.5157	-0.04028	0.5913
TNF-α	-0.13779	0.0593	-0.04890	0.5063	-0.05701	0.4396	-0.06591	0.3794
TNF-β	-0.13902	0.0571	-0.02514	0.7327	-0.02563	0.7284	-0.04689	0.5319
TRAIL	-0.04936	0.5012	0.04890	0.5063	0.05850	0.4277	0.04575	0.5419
VCAM1	0.04287	0.5591	-0.00337	0.9635	-0.00327	0.9646	-0.00245	0.9739
VEGF	-0.03167	0.6661	0.03041	0.6795	0.03348	0.6501	0.02895	0.6996

Table S2. Regression analysis results per "Secondary analysis: Fatigue duration" section of main text for assessment of trend in mean cytokine levels (pMFI) in ME/CFS patients across years of fatigue duration and sequence of mild, moderate (Mod), and severe (Sev) disease. With primary and secondary regression models otherwise identical, the secondary analysis was able to isolate the effect of fatigue duration (shown in lower half of table as "Fat. Dur."). P-values are for the significance of the trend and shown before and after adjustment for multiple comparisons (controlling for false discovery rate [FDR] at 5%). Statistically significant ($p < 0.05$) results are in bold. All results shown in this table are adjusted for age, sex, race and nonspecific binding.

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Cytokine	Hypothesis	Unadjusted p-value	FDR-adjusted p-value	Cytokine	Hypothesis	Unadjusted p-value	FDR-adjusted p-value
CCL2	LINEAR	0.0830	0.1453	IL-10	LINEAR	0.2183	0.2840
CCL3	LINEAR	0.2975	0.3669	IL-12p40	LINEAR	0.0918	0.1453
CCL4	LINEAR	0.4364	0.4613	IL-12p70	LINEAR	**0.0060**	**0.0267**
CCL5	LINEAR	0.3363	0.3770	IL-13	LINEAR	**0.0060**	**0.0267**
CCL7	LINEAR	0.0942	0.1453	IL-15	LINEAR	0.0869	0.1453
CCL11	LINEAR	**0.0079**	**0.0267**	IL-17	LINEAR	0.4788	0.4788
CD40L	LINEAR	0.5913	0.5913	IL-17F	LINEAR	**0.0088**	**0.0267**
CXCL1	LINEAR	**0.0246**	0.0536	Leptin	LINEAR	**0.0097**	**0.0267**
CXCL5	LINEAR	0.8410	0.8410	LIF	LINEAR	**0.0049**	**0.0267**
CXCL9	LINEAR	0.3548	0.3861	M-CSF	LINEAR	0.0743	0.1446
CXCL10	LINEAR	**0.0078**	**0.0267**	NGF	LINEAR	**0.0034**	**0.0267**
FASL	LINEAR	0.6538	0.6538	PAI-1	LINEAR	0.7690	0.7690
FGF-basic	LINEAR	0.6926	0.6926	PDGF-BB	LINEAR	0.2014	0.2840
G-CSF	LINEAR	**0.0101**	**0.0267**	Resistin	LINEAR	0.8787	0.8787
GM-CSF	LINEAR	**0.0018**	**0.0267**	SCF	LINEAR	**0.0215**	0.0531
HGF	LINEAR	0.3238	0.3770	TGF-α	LINEAR	**0.0388**	0.0797
ICAM1	LINEAR	0.7546	0.7546	TGF-β	LINEAR	0.8128	0.8128
IFN-α	LINEAR	0.5221	0.5221	TNF-α	LINEAR	0.2186	0.2840
IFN-β	LINEAR	0.2226	0.2840	TNF-β	LINEAR	0.5363	0.5363
IFN-γ	LINEAR	**0.0057**	**0.0267**	TRAIL	LINEAR	0.5039	0.5039

Cytokine	Hypothesis	Unadjusted p-value	FDR-adjusted p-value	Cytokine	Hypothesis	Unadjusted p-value	FDR-adjusted p-value
IL-1RA	LINEAR	0.9403	0.9403	VCAM1	LINEAR	0.3337	0.3770
IL-1α	LINEAR	**0.0238**	0.0536	VEGF	LINEAR	0.6472	0.6472
IL-1β	LINEAR	0.7385	0.7385	CCL2	NONLINEAR	0.8795	0.9169
IL-2	LINEAR	0.0840	0.1453	CCL3	NONLINEAR	0.5087	0.8063
IL-4	LINEAR	**0.0100**	**0.0267**	CCL4	NONLINEAR	1.0000	1.0000
IL-5	LINEAR	**0.0055**	**0.0267**	CCL5	NONLINEAR	0.3089	0.8063
IL-6	LINEAR	0.1178	0.1743	CCL7	NONLINEAR	0.9273	0.9466
IL-7	LINEAR	**0.0006**	**0.0222**	CCL11	NONLINEAR	0.5985	0.8063
IL-8	LINEAR	0.4647	0.4776	CD40L	NONLINEAR	1.0000	1.0000
CXCL1	NONLINEAR	0.1749	0.8008	TGF-α	NONLINEAR	0.2224	0.8008
CXCL5	NONLINEAR	0.6573	0.8476	TGF-β	NONLINEAR	0.8337	0.9078
CXCL9	NONLINEAR	**0.0397**	0.4858	TNF-α	NONLINEAR	1.0000	1.0000
CXCL10	NONLINEAR	0.5620	0.8063	TNF-β	NONLINEAR	0.3266	0.8063
FASL	NONLINEAR	0.7004	0.8799	TRAIL	NONLINEAR	0.8003	0.9078
FGF-basic	NONLINEAR	0.5721	0.8063	VCAM1	NONLINEAR	0.8061	0.9078
G-CSF	NONLINEAR	0.1846	0.8008	VEGF	NONLINEAR	0.4380	0.8063
GM-CSF	NONLINEAR	0.3390	0.8063	CCL2	Fat. Dur.	0.6524	1.0000
HGF	NONLINEAR	0.8208	0.9078	CCL3	Fat. Dur.	0.3995	1.0000
ICAM1	NONLINEAR	**0.0004**	**0.0105**	CCL4	Fat. Dur.	0.4190	1.0000
IFN-α	NONLINEAR	0.1242	0.8008	CCL5	Fat. Dur.	0.1515	1.0000
IFN-β	NONLINEAR	0.4775	0.8063	CCL7	Fat. Dur.	0.6761	1.0000
IFN-γ	NONLINEAR	0.4520	0.8063	CCL11	Fat. Dur.	0.6684	1.0000
IL-1RA	NONLINEAR	**0.0297**	0.4845	CD40L	Fat. Dur.	0.6337	1.0000
IL-1α	NONLINEAR	0.0650	0.6365	CXCL1	Fat. Dur.	0.9108	1.0000
IL-1β	NONLINEAR	0.4529	0.8063	CXCL5	Fat. Dur.	0.3267	1.0000
IL-2	NONLINEAR	0.3780	0.8063	CXCL9	Fat. Dur.	**0.0181**	0.8669
IL-4	NONLINEAR	0.5429	0.8063	CXCL10	Fat. Dur.	0.1424	1.0000

Cytokine	Hypothesis	Unadjusted p-value	FDR-adjusted p-value	Cytokine	Hypothesis	Unadjusted p-value	FDR-adjusted p-value
IL-5	NONLINEAR	0.5807	0.8063	FASL	Fat. Dur.	0.6158	1.0000
IL-6	NONLINEAR	0.5098	0.8063	FGF-basic	Fat. Dur.	0.7815	1.0000
IL-7	NONLINEAR	0.7777	0.9078	G-CSF	Fat. Dur.	0.7277	1.0000
IL-8	NONLINEAR	0.2288	0.8008	GM-CSF	Fat. Dur.	0.5156	1.0000
IL-10	NONLINEAR	0.5967	0.8063	HGF	Fat. Dur.	0.5086	1.0000
IL-12p40	NONLINEAR	0.0992	0.8008	ICAM1	Fat. Dur.	1.0000	1.0000
IL-12p70	NONLINEAR	0.5569	0.8063	IFN-α	Fat. Dur.	0.4678	1.0000
IL-13	NONLINEAR	0.8083	0.9078	IFN-β	Fat. Dur.	0.9301	1.0000
IL-15	NONLINEAR	0.2975	0.8063	IFN-γ	Fat. Dur.	0.4993	1.0000
IL-17	NONLINEAR	0.1588	0.8008	IL-1RA	Fat. Dur.	0.5574	1.0000
IL-17F	NONLINEAR	0.1982	0.8008	IL-1α	Fat. Dur.	**0.0340**	0.8669
Leptin	NONLINEAR	0.6089	0.8063	IL-1β	Fat. Dur.	1.0000	1.0000
LIF	NONLINEAR	0.3174	0.8063	IL-2	Fat. Dur.	0.5199	1.0000
M-CSF	NONLINEAR	0.2840	0.8063	IL-4	Fat. Dur.	0.7070	1.0000
NGF	NONLINEAR	0.1673	0.8008	IL-5	Fat. Dur.	0.5271	1.0000
PAI-1	NONLINEAR	0.8705	0.9169	IL-6	Fat. Dur.	0.0736	1.0000
PDGF-BB	NONLINEAR	0.4404	0.8063	IL-7	Fat. Dur.	0.8621	1.0000
Resistin	NONLINEAR	**0.0002**	**0.0091**	IL-8	Fat. Dur.	0.6402	1.0000
SCF	NONLINEAR	0.3805	0.8063	IL-10	Fat. Dur.	0.4233	1.0000
IL-12p40	Fat. Dur.	0.9113	1.0000	PDGF-BB	Fat. Dur.	0.7215	1.0000
IL-12p70	Fat. Dur.	0.7362	1.0000	RESISTIN	Fat. Dur.	0.7434	1.0000
IL-13	Fat. Dur.	0.8929	1.0000	SCF	Fat. Dur.	0.5278	1.0000
IL-15	Fat. Dur.	0.7818	1.0000	TGF-α	Fat. Dur.	1.0000	1.0000
IL-17	Fat. Dur.	0.6491	1.0000	TGF-β	Fat. Dur.	0.5935	1.0000
IL-17F	Fat. Dur.	0.7867	1.0000	TNF-α	Fat. Dur.	0.6024	1.0000
LEPTIN	Fat. Dur.	1.0000	1.0000	TNF-β	Fat. Dur.	0.6734	1.0000
LIF	Fat. Dur.	0.7125	1.0000	TRAIL	Fat. Dur.	0.5698	1.0000

Cytokine	Hypothesis	Unadjusted p-value	FDR-adjusted p-value	Cytokine	Hypothesis	Unadjusted p-value	FDR-adjusted p-value
M-CSF	Fat. Dur.	0.8525	1.0000	VCAM1	Fat. Dur.	0.8256	1.0000
NGF	Fat. Dur.	0.8895	1.0000	VEGF	Fat. Dur.	0.2373	1.0000
PAI-1	Fat. Dur.	0.4206	1.0000				

Table S3. Regression of cytokine pMFI on Short (≤ 3 years) vs. Long (> 3 years) Fatigue Duration and Disease Severity (51). Analysis was adjusted for covariates of age, sex, race and nonspecific binding (CHEX4 pMFI).

Test	Cytokine	Unadjusted p-value	Adjusted p-value
Long v Short Duration	CCL2	0.9481	0.9889
Long v Short Duration	CCL3	0.3305	0.9889
Long v Short Duration	CCL4	0.6272	0.9889
Long v Short Duration	CCL5	0.1563	0.9889
Long v Short Duration	CCL7	0.6980	0.9889
Long v Short Duration	CCL11	0.8058	0.9889
Long v Short Duration	CD40L	0.5735	0.9889
Long v Short Duration	CXCL1	0.9704	0.9889
Long v Short Duration	CXCL5	0.1243	0.9889
Long v Short Duration	CXCL9	0.6672	0.9889
Long v Short Duration	CXCL10	0.3970	0.9889
Long v Short Duration	FASL	0.1272	0.9889
Long v Short Duration	FGF-basic	0.7173	0.9889
Long v Short Duration	G-CSF	0.9622	0.9889
Long v Short Duration	GM-CSF	0.2997	0.9889
Long v Short Duration	HGF	0.9889	0.9889
Long v Short Duration	ICAM1	0.7043	0.9889
Long v Short Duration	IFN-α	0.3196	0.9889
Long v Short Duration	IFN-β	0.7312	0.9889
Long v Short Duration	IFN-γ	0.8208	0.9889
Long v Short Duration	IL-1RA	0.1940	0.9889
Long v Short Duration	IL-1α	0.5932	0.9889
Long v Short Duration	IL-1β	0.2113	0.9889
Long v Short Duration	IL-2	0.4793	0.9889
Long v Short Duration	IL-4	0.8655	0.9889
Long v Short Duration	IL-5	0.3282	0.9889
Long v Short Duration	IL-6	0.1513	0.9889

Test	Cytokine	Unadjusted p-value	Adjusted p-value
Long v Short Duration	IL-7	0.5297	0.9889
Long v Short Duration	IL-8	0.6708	0.9889
Long v Short Duration	IL-10	0.2120	0.9889
Long v Short Duration	IL-12p40	0.8907	0.9889
Long v Short Duration	IL-12p70	0.3724	0.9889
Long v Short Duration	IL-13	0.3284	0.9889
Long v Short Duration	IL-15	0.6507	0.9889
Long v Short Duration	IL-17	0.6457	0.9889
Long v Short Duration	IL-17F	0.7033	0.9889
Long v Short Duration	LEPTIN	0.5283	0.9889
Long v Short Duration	LIF	0.7002	0.9889
Long v Short Duration	M-CSF	0.7208	0.9889
Long v Short Duration	NGF	0.8947	0.9889
Long v Short Duration	PAI-1	0.1168	0.9889
Long v Short Duration	PDGF-BB	0.7709	0.9889
Long v Short Duration	RESISTIN	0.9349	0.9889
Long v Short Duration	SCF	0.9510	0.9889
Long v Short Duration	TGF-α	0.9106	0.9889
Long v Short Duration	TGF-β	0.2934	0.9889
Long v Short Duration	TNF-α	0.6152	0.9889
Long v Short Duration	TNF-β	0.9053	0.9889
Long v Short Duration	TRAIL	0.5866	0.9889
Long v Short Duration	VCAM1	0.5785	0.9889
Long v Short Duration	VEGF	0.8199	0.9889

Table S4. Regression of cytokine pMFI over the two-dimensional distribution of total scores from the MFI-20 (disease severity) and fatigue duration in years. A statistically significant test of the duration by severity interaction would provide evidence that the relationship between mean cytokine expression and disease severity changes with duration of disease. “Linear Fatigue Duration” is linear (straight line) association with fatigue duration. “Complex Fatigue Duration” includes nonlinear associations with fatigue duration as well as fatigue duration by severity interaction. Analysis was adjusted for covariates of age, sex, race and nonspecific binding.

Test	Cytokine	Unadjusted p-value	Adjusted p-value
Linear Fatigue Duration	CCL2	0.4819	0.9530
Linear Fatigue Duration	CCL3	0.2539	0.9530
Linear Fatigue Duration	CCL4	0.3120	0.9530
Linear Fatigue Duration	CCL5	0.2892	0.9530
Linear Fatigue Duration	CCL7	0.4919	0.9530
Linear Fatigue Duration	CCL11	0.5665	0.9530
Linear Fatigue Duration	CD40L	0.4220	0.9530
Linear Fatigue Duration	CXCL1	0.7194	0.9530
Linear Fatigue Duration	CXCL5	0.2746	0.9530
Linear Fatigue Duration	CXCL9	0.0097	0.4922
Linear Fatigue Duration	CXCL10	0.0779	0.9530
Linear Fatigue Duration	FASL	0.8301	0.9530
Linear Fatigue Duration	FGF-basic	0.9093	0.9530
Linear Fatigue Duration	G-CSF	0.5488	0.9530
Linear Fatigue Duration	GM-CSF	0.5381	0.9530
Linear Fatigue Duration	HGF	0.3198	0.9530
Linear Fatigue Duration	ICAM1	0.8954	0.9530
Linear Fatigue Duration	IFN-α	0.6073	0.9530
Linear Fatigue Duration	IFN-β	0.8195	0.9530
Linear Fatigue Duration	IFN-γ	0.3356	0.9530
Linear Fatigue Duration	IL-1RA	0.3051	0.9530
Linear Fatigue Duration	IL-1α	0.0579	0.9530

Test	Cytokine	Unadjusted p-value	Adjusted p-value
Linear Fatigue Duration	IL-1β	0.8151	0.9530
Linear Fatigue Duration	IL-2	0.2528	0.9530
Linear Fatigue Duration	IL-4	0.6697	0.9530
Linear Fatigue Duration	IL-5	0.5217	0.9530
Linear Fatigue Duration	IL-6	0.0852	0.9530
Linear Fatigue Duration	IL-7	0.9205	0.9530
Linear Fatigue Duration	IL-8	0.9367	0.9530
Linear Fatigue Duration	IL-10	0.3250	0.9530
Linear Fatigue Duration	IL-12p40	0.6933	0.9530
Linear Fatigue Duration	IL-12p70	0.8464	0.9530
Linear Fatigue Duration	IL-13	0.9020	0.9530
Linear Fatigue Duration	IL-15	0.8169	0.9530
Linear Fatigue Duration	IL-17	0.9191	0.9530
Linear Fatigue Duration	IL-17F	0.6079	0.9530
Linear Fatigue Duration	LEPTIN	0.7625	0.9530
Linear Fatigue Duration	LIF	0.5539	0.9530
Linear Fatigue Duration	M-CSF	0.8944	0.9530
Linear Fatigue Duration	NGF	0.7961	0.9530
Linear Fatigue Duration	PAI-1	0.6091	0.9530
Linear Fatigue Duration	PDGF-BB	0.9530	0.9530
Linear Fatigue Duration	RESISTIN	0.4421	0.9530
Linear Fatigue Duration	SCF	0.2817	0.9530
Linear Fatigue Duration	TGF-α	0.8651	0.9530
Linear Fatigue Duration	TGF-β	0.5000	0.9530
Linear Fatigue Duration	TNF-α	0.6383	0.9530
Linear Fatigue Duration	TNF-β	0.5181	0.9530
Linear Fatigue Duration	TRAIL	0.6939	0.9530
Linear Fatigue Duration	VCAM1	0.9088	0.9530
Linear Fatigue Duration	VEGF	0.3291	0.9530
Complex Fatigue Duration	CCL2	0.3085	0.6910
Complex Fatigue Duration	CCL3	0.7197	0.7809

Test	Cytokine	Unadjusted p-value	Adjusted p-value
Complex Fatigue Duration	CCL4	0.5114	0.6929
Complex Fatigue Duration	CCL5	0.1858	0.6910
Complex Fatigue Duration	CCL7	0.5162	0.6929
Complex Fatigue Duration	CCL11	0.5832	0.7082
Complex Fatigue Duration	CD40L	0.6737	0.7635
Complex Fatigue Duration	CXCL1	0.3079	0.6910
Complex Fatigue Duration	CXCL5	0.5029	0.6929
Complex Fatigue Duration	CXCL9	0.2093	0.6910
Complex Fatigue Duration	CXCL10	0.5061	0.6929
Complex Fatigue Duration	FASL	0.2735	0.6910
Complex Fatigue Duration	FGF-basic	0.5692	0.7080
Complex Fatigue Duration	G-CSF	0.5590	0.7080
Complex Fatigue Duration	GM-CSF	0.0781	0.6910
Complex Fatigue Duration	HGF	0.2641	0.6910
Complex Fatigue Duration	ICAM1	0.6184	0.7335
Complex Fatigue Duration	IFN-α	0.7366	0.7826
Complex Fatigue Duration	IFN-β	0.8399	0.8567
Complex Fatigue Duration	IFN-γ	0.3273	0.6910
Complex Fatigue Duration	IL-1RA	0.6950	0.7706
Complex Fatigue Duration	IL-1α	0.3978	0.6929
Complex Fatigue Duration	IL-1β	0.8742	0.8742
Complex Fatigue Duration	IL-2	0.3877	0.6929
Complex Fatigue Duration	IL-4	0.5006	0.6929
Complex Fatigue Duration	IL-5	0.1604	0.6910
Complex Fatigue Duration	IL-6	0.1387	0.6910
Complex Fatigue Duration	IL-7	0.2052	0.6910
Complex Fatigue Duration	IL-8	0.2308	0.6910
Complex Fatigue Duration	IL-10	0.0480	0.6910
Complex Fatigue Duration	IL-12p40	0.2677	0.6910
Complex Fatigue Duration	IL-12p70	0.1361	0.6910

Test	Cytokine	Unadjusted p-value	Adjusted p-value
Complex Fatigue Duration	IL-13	0.1962	0.6910
Complex Fatigue Duration	IL-15	0.2649	0.6910
Complex Fatigue Duration	IL-17	0.2697	0.6910
Complex Fatigue Duration	IL-17F	0.4329	0.6929
Complex Fatigue Duration	LEPTIN	0.6580	0.7627
Complex Fatigue Duration	LIF	0.4568	0.6929
Complex Fatigue Duration	M-CSF	0.3317	0.6910
Complex Fatigue Duration	NGF	0.4783	0.6929
Complex Fatigue Duration	PAI-1	0.1380	0.6910
Complex Fatigue Duration	PDGF-BB	0.2932	0.6910
Complex Fatigue Duration	RESISTIN	0.4626	0.6929
Complex Fatigue Duration	SCF	0.2427	0.6910
Complex Fatigue Duration	TGF-α	0.3149	0.6910
Complex Fatigue Duration	TGF-β	0.4869	0.6929
Complex Fatigue Duration	TNF-α	0.2047	0.6910
Complex Fatigue Duration	TNF-β	0.3547	0.6929
Complex Fatigue Duration	TRAIL	0.8003	0.8329
Complex Fatigue Duration	VCAM1	0.3387	0.6910
Complex Fatigue Duration	VEGF	0.5574	0.7080